



Patent Application
Attorney's Docket No. 010055-134

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of

Simon C. BURTON et al.

Application No.: 08/468,610

Filed: June 6, 1995

For: CHROMATOGRAPHIC RESINS AND
METHODS FOR USING SAME

Group Art Unit: 1651

Examiner: Jon P. Weber, Ph.D.

DECLARATION UNDER 37 C.F.R. § 1.132

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

I, Steven M. CRAMER, do hereby declare:

1. THAT, I have received a Bachelor of Science in Chemical Engineering from Brown University in 1978, a Master of Science in Chemical Engineering from Yale University in 1982, and a Ph.D. in Chemical Engineering from Yale University in 1986.

2. THAT, I am an employee of Rensselaer Polytechnic Institute (hereinafter "Rensselaer"), where I have worked since 1986. I am a chemical and biomedical engineer by training and am currently a Professor of Chemical Engineering at Rensselaer. Since coming to Rensselaer in 1986, I have worked in and have been recognized as an expert in the fields of Chromatographic Bioprocessing and Separation Science. I am a co-inventor of several issued patents in the field of protein chromatography, and I have published over 70 articles including many in this field. I have also developed mathematical models of protein chromatography which allow accurate prediction of effluent profiles in process-scale separations. I also have extensive experience in membrane separations, enzyme

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technology, and environmental separations. I am also an editor of the journal Separation Science and Technology and a fellow of the American Institute for Medical and Biological Engineering.

3. THAT, a copy of my Curriculum Vitae is attached hereto as Appendix A.

4. THAT, I have reviewed and am familiar with the contents of U.S. Patent Application Serial No. 08/468,610 (hereinafter "'610 patent application") including the currently pending claims.

5. THAT, the invention in the '610 patent application relates to complexes of chromatographic resins with proteins and peptides. In particular, the chromatographic resins are useful for the binding of a target protein or peptide from an aqueous medium that has either a high or low ionic strength. Central to the claimed invention is the use of an electrostatically uncharged resin at the pH where the target protein or peptide is bound to the resin which has a pH in the range of from 5 to 9 when the aqueous medium has either a high or low ionic strength. In addition, the resin is selected such that it contains an electrostatic charge at the pH where the protein or peptide is desorbed from the resin, wherein the desorption occurs by a change in the pH from the binding pH.

6. THAT, I have reviewed and am familiar with the Office Action dated September 28, 2001. I have also reviewed and am familiar with the Examiner's rejection of the claims alleging that such claims are purportedly anticipated by Boardman et al., *Nature*, 171:208-210 (1953) (hereinafter "Boardman").

7. THAT, I have reviewed and am familiar with the Examiner's rejection of the claims alleging that such claims are purportedly obvious over Boardman, Sasaki et al., *J. Biochem.*, 86:1537-1548 (1979) ("Sasaki 1979") and Sasaki et al., *J. Biochem.*, 91:1551-1561 (1982) ("Sasaki 1982") in view of Kunin, *Ion Exchange Resins*, 34-39 (John Wiley &

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Sons, Inc., Interscience 1958) ("Kunin"), Topp et al., *J. Chem. Soc.*, Pt. 2:3299-3303 (1949) ("Topp"), Kitchener, Ion Exchangers In Organic and Biochemistry, 63-64 (Calmon and Kressman eds., Interscience Publishers, Inc. 1957) ("Kitchener") and Guthrie, Ion Exchangers In Organic and Biochemistry, 558-559 (Calmon and Kressman eds., Interscience Publishers, Inc. 1957) ("Guthrie").

8. THAT, I have reviewed and am familiar with the Boardman, Sasaki 1979, Sasaki 19872, Kunin, Topp, Kitchener and Guthrie references cited in the Office Action of September 28, 2001.

9. THAT, I have reviewed and am familiar with the Reply of January 28, 2002, filed in response to this Office Action which includes the Declaration under 37 C.F.R. § 1.132 by Nathaniel T. Becker, one of the joint inventors of the subject matter disclosed and claimed in the present application (hereinafter the "Becker Declaration"). The Becker Declaration was filed as an attachment to the Reply of January 28, 2002.

10. THAT, I have reviewed and am familiar with the product literature regarding the synthetic cation exchange resin, Amberlite IRC-50, provided by the manufacturer, Rohm and Haas Co. (2000) (hereinafter "Rohm and Haas"). A copy of Rohm and Haas was attached to the Becker Declaration that was filed with the Reply of January 28, 2002.

11. THAT, I have reviewed and am familiar with the Declaration under 37 C.F.R. § 1.132 by Simon C. Burton, one of the joint inventors of the subject matter disclosed and claimed in the present application (hereinafter the "Burton Declaration"). I have also reviewed and am familiar with the experimental carboxylate titration curve data discussed in the Burton Declaration.

12. THAT, after reviewing these various documents, I agree with the opinions set forth in the Becker Declaration and the Burton Declaration with regard to the Amberlite

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IRC-50 resin. In particular, I agree that the Amberlite IRC-50 resin is a weakly acidic cation exchange resin which would become fully protonated at a pH of 2.5 to 4.0 depending on the buffer salts present. Based on the titration data supplied by the manufacturer in the reference by Kunin, in the Rohm and Haas product literature, and in the data generated by Burton, the IRC-50 resins remain charged at pH 5 and are not fully protonated until pH less than 4.0. This is clearly demonstrated in the Rohm and Haas product literature. In fact, it is my opinion that typical weak cation exchangers used in the biotechnology industry (e.g., CM-Sephadex) have pKa's in the 3.5 to 4 range, thus requiring a low pH to become fully protonated and uncharged. Therefore, it is my opinion that the prior art referenced above fails to teach or suggest binding of proteins to uncharged weak cation exchangers in the pH range of 5 to 9 at high or low ionic strength.

13. THAT, it is my experience that derivatization chemistry can often result in several different types of charged groups on an ion exchange resin due to different microenvironments. Thus, it is good practice to use experimental titration data rather than theoretical calculations to determine the charged state of ion exchange resins. In this regard, I agree with the titration curve data discussed in the Burton Declaration relating to Amberlite and acetic acid. This carboxylate titration data found in the Burton Declaration confirms that the pKa is irrelevant except for theoretical calculations of the percentage of protonated carboxyl groups using the Henderson-Hasselbalch equation. However, these theoretical considerations do not take the place of experimental data. As shown from the titration curve in the Burton Declaration and in those from the above-cited references, in particular Kunin, Kitchener, Rohm and Haas, the experimental titration curves do not fit those calculated theoretically. Instead, the titration range is broader, probably due to heterogeneity and neighboring group effects in a polyvalent species such as an ion exchanger. Thus, I believe theoretical pKa data should be ignored in favor of experimental titration data.

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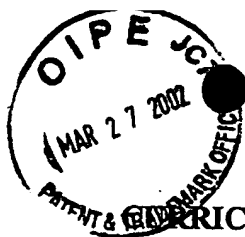
14. I further declare that all statements made herein of my own knowledge are true and all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: 3/27/02

Signed: 
Steven M. Cramer

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APPENDIX A



RESUME/CURRICULUM VITAE FOR STEVEN M. CRAMER

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(518) 377-9074

Birthdate: 2/11/56

Business Address

Department of Chemical Engineering
Rensselaer Polytechnic Institute
Troy, NY 12180-3590
(518) 276-6198

Educational Background

1986 PhD in Chemical Engineering, Yale University, New Haven, Connecticut

1982 Master of Science in Chemical Engineering, Yale University, New Haven, Connecticut

1978 Bachelor of Science in Biomedical Engineering, Brown University, Providence, Rhode Island

Professional Experience

5/95-Present: Professor of Chemical Engineering, Department of Chemical Engineering, Rensselaer Polytechnic Institute, Troy, New York.

7/93-5/95: Isermann Associate Professor of Chemical Engineering, Department of Chemical Engineering, Rensselaer Polytechnic Institute, Troy, New York.

1/90-7/93: Associate Professor of Chemical Engineering, Department of Chemical Engineering, Rensselaer Polytechnic Institute, Troy, New York.

9/86 - 1/90: Isermann Assistant Professor of Chemical Engineering, Department of Chemical Engineering, Rensselaer Polytechnic Institute, Troy, New York.

9/78 - 8/81: Research Engineer, Amicon Corporation, Lexington, Massachusetts.

Honors

6/01	Co-Chair of ACS Conference on Recovery of Biological Products
7/99	Chair of Gordon Conference on Reactive Polymers and Ion Exchange
12/96	Elected a Fellow of American Institute for Medical and Biological Engineering
3/96	Executive Editor: Separation Science and Technology
5/95	Special Associate Editor: Biotechnology and Bioengineering
5/90	Early Career Award, Rensselaer Polytechnic Institute.

3/89 Presidential Young Investigator, National Science Foundation.
3/89 Lilly Teaching Fellow Award, Rensselaer Polytechnic Institute.
9/87 Dow Chemical Company Excellence in Teaching Award.
9/86-Present Editorial Board, Isolation and Purification

Professional Activities

Membership in: American Chemical Society, American Institute of Chemical Engineers, American Association for the Advancement of Science.

Co-Chair Biotechnology Secretariat Program for 1994 San Diego ACS Meeting.
Executive Committee, Biotechnology Secretariat, American Chemical Society.
Executive Committee, I & EC Division, American Chemical Society.
Executive Committee, Separation Science and Technology Subdivision, American Chemical Society.

Reviewer for journals and agencies

National Science Foundation	Trends in Analytical Chemistry
Journal of Chromatography	Chemical Engineering Communications
Biotechnology Journal	Biotechnology Progress
Reactive Polymers	AIChE Journal
Biotechnology and Bioengineering	University of Queensland (PhD Thesis)
I&EC Research	Yale University (PhD Thesis)
Preparative Chromatography	American Scientist
Chemical Engineering Science	Separations Technology
Proceedings of National Academy of Sciences	Chemistry of Materials
M.I.T. (Ph.D. Thesis)	Biotechnology Advances
Health Effects Institute	National Institute of Health
Nature	Computers in Chemical Engineering
	Journal of Colloid and Interface Science

Involved in following activities at Rensselaer

Bioseparations and Biophysics Centers, Chair of Chemical Engineering Curriculum Committee, Engineering School Committee on Reward Structure.

Consulting

Served as a consultant to Millipore Corporation, H. R. Parrs Associates, Boehringer Labs, NASA, MARS Corporation, Allied Signal, National Institute of Health, Health Effects Institute, Merck, Protein Design Labs, Chiral Technologies, Genentech, IGEN, DYAX, Regeneron, ISIS, Merck, Amersham Pharmacia, SACHEM, Chiron, Biogen, Novo Nordisk, Antigenics, Cabot, Tanical Therapeutics.

Sessions Chaired at Meetings

Chaired a session on proteomics at ISPPP meeting (2001).

Chaired a workgroup on a NSF-DOE retreat on combinatorial technology, Boston, Ma. (1997).

Novel Bioseparations Processes, ACS 96 Spring Meeting, New Orleans, LA. (1996)

Chromatographic Bioprocessing, Recovery of Biological Products VIII, Tucson, Arizona. (1996)

Preparative Chromatography, Prep 95, Washington, DC. (1995)

Mass Transport in Chromatographic Systems, International Society of Proteins, Polynucleotides, and Peptides, Boston, MA. (1995)

Biotechnology, Pacifichem, Hawaii. (1995)

Bioseparations, ACS Meeting, San Diego, CA (1994).

Protein Separations, Gordon Conference on Separation and Purification, New London, NH (1994).

Bioseparations, ACS Meeting, Denver, CO (1993).

Bioseparations, AIChE Meeting, St. Louis, MO (1993).

Sessions Co-Chairman, "Transport Processes in Bioseparations Systems (I and II), AIChE Annual Meeting, Miami, FL (1992).

Session co-chairman, "Chromatographic Engineering in Bioseparations", AIChE Annual Meeting, Los Angeles, CA, 1991.

Session chairman, "Mathematical Models of Preparative Chromatography", 8th International Symposium on Preparative Chromatography", Arlington, VA, 1991.

Session co-chairman, "Novel Engineering Approaches to Bioseparations", ACS National Meeting, Washington, D.C., 1990.

Session chairman, "Chromatography", Gordon Conference on Separations and Purifications, New London, NH, 1990.

Session co-chairman. "Transport Processes in Bioseparation Systems", AIChE Annual Meeting, San Fransisco, 1989.

Session co-chairman. "Young Faculty Forum", AIChE Annual Meeting, Washington, 1988.

Session co-chairman. "Transport Processes in Bioseparation Systems" AIChE Annual Meeting, Washington, 1988.

Patents

US Patent # 5,478,924 "Displacement Chromatography of Proteins using Low Molecular Weight Displacers", S. M. Cramer, J. A. Moore, A. Kundu, Y. Li, G. Jayaraman. (1995).

US Patent # 5,606,033 "Displacement Chromatography of Proteins using Low Molecular Weight Anionic Displacers", S. M. Cramer, J. A. Moore, A. Kundu, Y. Li, G. Jayaraman. (1997).

US Patent # 6,239,262 "Low Molecular Weight Displacers for Protein Purificaiton in Hydrophobic Interaction and Reversed Phase Chromatographic Systems", S. M. Cramer, A. Shukla, and K. Sunasara. (2001).

"High Affinity, Low Molecular Weight Displacers for Oligonucleotide Purification", S. M. Cramer, A. Shukal, R. Deshmukh, J. Moore. Claims allowed, to be issued in early 2002.

Publications (Refereed Journal Articles)

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Sunasara, K. M.; Rupp, R. G. and Cramer, S. M., Purification of recombinant brain derived neurotrophic factor using reversed phase displacement chromatography, Biotechnol. Progr., 17 (5): 897-906, Sept. - Oct., 2001

Ghose, S. and Cramer, S. M., Characterization and modeling of monolithic stationary phases: application to preparative chromatography, J Chromatogr. A, 928 (1): 13-23 Aug. 31, 2001

Tugcu, N.; Deshmukh, R. R. and Sanghvi, Y. S., et al., Purification of an oligonucleotide at high column loading by high affinity, low-molecular-mass displacers, J Chromatogr. A, 923 (1-2): 65-73 July 20, 2001

Tugcu, N.; Moore, J. A. and Cramer, S. M., Synthesis and characterization of high-affinity, low molecular mass displacers for anion-exchange chromatography, submitted to *Industrial and Engineering Chemistry*.

Mazza, C. B.; Rege, K.; Breneman, C. M.; Dordick, J. S. and Cramer, S. M., High throughput screening and quantitative-structure efficacy relationship models of potential displacer molecules for ion exchange systems, submitted to *Biotechnology and Bioengineering*.

Tugcu, N.; Mazza, C.; Breneman, C.; Sanghvi, Y.; Moore, J. and Cramer, S. M., High throughput screening and quantitative structure efficacy relationship models for designing displacers for anti-sense oligonucleotide purification in anion-exchange systems, in press, *Separation Science and Technology*.

Tugcu, N.; Bae, S.; Moore, J. A. and Cramer, S. M., The role of the stationary phase on the dynamic affinity of various low-molecular-mass displacers, submitted to *J. of Chromatography*.

Kalra, A.; Tugcu, N.; Cramer, S. M.; and Garde, S., Salting-in and salting-out of hydrophobic solutes in aqueous salt solutions, *J. Phys. Chem. B*, 105 (27), 6380 -6386, 2001.

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Natarajan, V.; Bequette, B.W. and Cramer, S. M., Optimization of ion exchange displacement separations. I. Validation of an iterative scheme and its use as a methods development tool", *J Chromatogr. A*, 876 (1-2) 51-62 April 21, 2000

Natarajan, V. and Cramer, S. M., Optimization of ion exchange displacement separations. II. Comparison of displacement separation on various ion exchange resins, *J Chromatogr. A*, 876 (1-2) 63-73 April 21, 2000

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Kim, Y. and Cramer, S. M., Metal chelate displacement chromatography of proteins, *J. Chromatogr.* 549, 89-99, 1991.

Cramer, S. M., Displacement chromatography, *Nature* 351, 251-252, 1991.

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Cramer, S. M. and Subramanian, G., Recent advances in the theory and practice of displacement chromatography, *Sep. Purif. Methods*, 19 (1), 31-91, 1990.

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Wu, D.; Belfort, G. and Cramer, S. M., Enzymatic resolution with a multiphase membrane bioreactor: a theoretical analysis, *I & EC Research*, 29, 1612-1621, 1990.

Subramanian, G.; Phillips, M. W.; Jayaraman, G. and Cramer, S. M., Displacement chromatography of biomolecules with large particle diameter systems, *J. Chromatogr.*, 484, 225, 1989.

Subramanian, G. and Cramer, S. M., Displacement chromatography of proteins under elevated flow rate and crossing isotherm conditions, *Biotech. Progress* 5 (3), 1989.

Cramer, S. M. and Horvath, Cs., Peptide synthesis and deamidation with chemically modified immobilized carboxypeptidase Y., *Enzyme Microb. Technol.* 11 (2), 74, 1989.

Cramer, S. M. and Horvath, Cs., Peptide synthesis with immobilized carboxypeptidase Y, *Biotechnol. Bioeng.* 33, 344, 1989.

Phillips, M. W., Subramanian, G. and Cramer, S. M., A theoretical optimization of operating parameters in non-ideal displacement chromatography, *J. Chromatogr.*, 454, 1, 1988.

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